# Antidipsogenic Effect of Clonidine on Angiotensin II-, Hypertonic Saline-, Pilocarpine- and Dehydration-Induced Water Intakes[1]

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FREGLY, M. J., D. L. KELLEHER AND J. E. GREENLEAF. *Antidipsogenic effect of clonidine on angiotensin II-, hypertonic saline-, pilocarpine- and dehydration-induced water intakes.* BRAIN RES. BULL. 7(6) 661–664, 1981.—The dipsogenic responses of female rats to administration of angiotensin II (150 $\mu$g/kg b.w., IP), pilocarpine (3 mg/kg IP), hypertonic saline (1 M NaCl solution, 1% b.w.), and a 24 hour dehydration were attenuated by acute IP administration of graded doses of the central and peripheral $\alpha_2$-adrenergic agonist, clonidine. For all treatments except dehydration, clonidine inhibited significantly the dipsogenic response at the lowest dose used (6 $\mu$g/kg, IP). The first significant effect on dehydration-induced drinking required approximately a 4 fold higher dose (25 $\mu$g/kg, IP). Attenuation of the response to these dipsogenic stimuli by clonidine, suggests that its ability to stimulate $\alpha$-adrenergic receptors centrally may play an important role in its dipsogenic inhibitory activity.

Rats     Thirst     Drinking     $\alpha$-Adrenergic agonist     Parasympathomimetic agent

ACUTE administration of clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride), a central $\alpha$-adrenergic agonist, inhibits the drinking response of rats to acute administration of the $\beta$-adrenergic agonist, isoproterenol [7]. Drinking was inhibited in a dose-dependent fashion with the first significant effect occurring at the lowest dose used, i.e., 12 $\mu$g/kg of b.w., IP. The mechanism by which drinking was inhibited by clonidine was not ascertained in these studies. However, clonidine inhibits the secretion of renin by the kidneys, perhaps indirectly as a result of its central $\alpha$-adrenergic activity [15, 17, 22]. Clonidine also stimulates peripheral presynaptic $\alpha$-adrenoreceptors and could also affect renin secretion at this level [2, 16, 21]. In addition, clonidine may, by virtue of its central $\alpha$-adrenergic activity, inhibit the center or centers in the brain responsible for mediating drinking in the rat. If this latter hypothesis were correct, other types of experimentally induced drinking should also be attenuated since peripheral stimuli may initiate drinking by activation of centers in the brain which mediate this function [5]. Hence, the objective of these experiments was to determine whether clonidine had an antidipsogenic effect on angiotensin II-, hypertonic saline-, pilocarpine- and dehydration-induced water intakes.

## METHOD

Female rats of the Blue Spruce Farms (Sprague-Dawley) strain were used in the four studies reported here. The rats ranged in body weight from 180 to 300 g at the beginning of the experiment. All animals were kept in a thermoregulated (25±1°C) room illuminated from 6 a.m. to 6 p.m. Purina Laboratory Chow and tap water were provided ad lib. All drinking studies were performed in a quiet thermoregulated (25±1°C) room beginning at 9:30 a.m.

The data from all experiments were analyzed by a one-way analysis of variance [18]. Comparison between individual means was made by the $t$-test using the pooled variance from the analysis of variance [8].

*Experiment 1. Effect of Angiotensin II and Clonidine, Separately and in Combination, on Water Intake*

Twenty-four naive rats (180–220 g) were divided randomly into four equal groups. Group 1 received angiotensin II (150 $\mu$g/kg, IP) while the other 3 groups also received angiotensin II(150 $\mu$g/kg, IP) in combination with doses of 6, 12, and 24 $\mu$g clonidine [4]/kg, IP, respectively. After administration of the drugs, each rat was placed in an individual
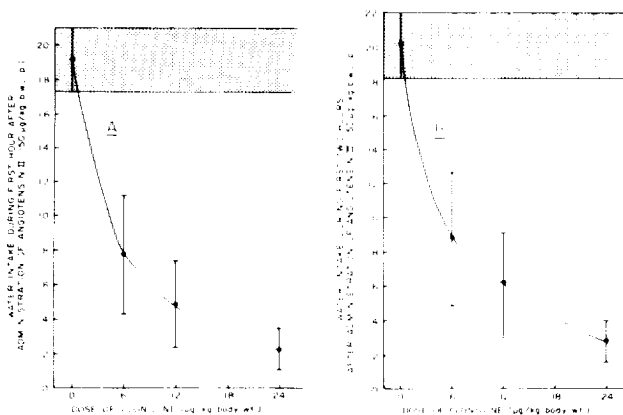
FIG. 1. Water intakes (±SE) of rats during the first (A) and second (B) hours after administration of angiotensin II(150 μg/kg IP) alone or in combination with 6, 12, and 24 μg clonidine/kg of b.w., IP are shown. Shaded bar at the top of this and all succeeding figures represents water intake (±1SE) induced by the dipsogen alone.

FIG. 2. Water intakes (±SE) of rats during the first (A) and second (B) hours after administration of a hypertonic saline load (1 M NaCl. 1 ml/100 g b.w., IP) alone (shaded bar at top of each figure) and hypertonic saline in combination with 6, 12 and 24 μg clonidine/kg of body weight. IP are shown.

stainless steel metabolism cage and given a preweighed water bottle containing distilled water [12]. Water intake was measured at 1 and 2 hours. No food was available to the rats during the study.

*Experiment 2. Effect of Clonidine on Water Intake Following Administration of a Hypertonic Load of NaCl Solution*

Twenty-four naive rats (230–260 g) were lightly anesthetized with ether to induce reflex emptying of the bladder, weighed and administered IP 1 M NaCl solution (1 ml/100 g b.w. warmed to 37°C). The animals were divided randomly into 4 equal groups. Three of the groups (18 rats) were administered hypertonic saline and received at the same time either 6, 12 or 24 μg of clonidine/kg IP. The fourth group received only hypertonic saline and an equal volume of vehicle. Each rat was then placed in an individual stainless steel metabolism cage and the drinking response was measured as described in Experiment 1.

*Experiment 3. Effect of Clonidine on Water Intake by Rats Administered the Parasympathomimetic Agent, Pilocarpine*

Eighteen naive rats were divided into 3 equal groups, all of which received 3 mg pilocarpine/kg, b.w. IP. This dose was chosen because it produced the maximal drinking response in an earlier study [6]. At the same time, two of the groups received 6 and 12 μg clonidine/kg IP, respectively. The third group received an equal volume of distilled water, the vehicle used to dissolve the clondine. Each rat was then placed in an individual stainless steel metabolism cage. The study was carried out as described in Experiment 1.

*Experiment 4. Effect of Clonidine on Water Intake by Rats Dehydrated for 24 Hours*

Twenty-four naive rats were weighed and then dehydrated, with food available, for 24 hours prior to the experiment. At the end of this time, all rats were again weighed and 2 groups (8 rats each) were administered IP either 6 or 12 μg clonidine/kg, respectively. The third group served as con-
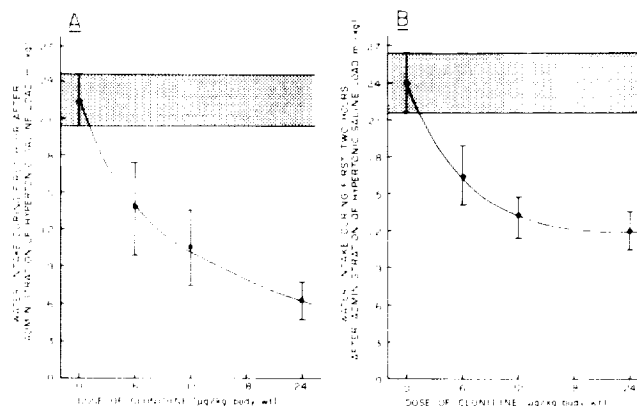
trols and received IP an equal volume of distilled water. Each rat was then placed into an individual metabolism cage and water intake measured as described in Experiment 1. A second study was done identically to the first excepting that two of the groups were administered 25 and 50 μg clonidine/kg, IP, respectively.

## RESULTS

*Experiment 1*

Water intakes of all groups during the first and second hours after administration of graded doses of clonidine given in combination with 150 μg angiotensin II/kg are shown in Fig. 1. The effect of increasing doses of clondine administered in combination with angiotensin II was to inhibit the angiotensin-stimulated drinking response in a dose-dependent fashion. The first significant ($p<0.05$) effect occurred at the lowest dose of clonidine used (6 μg/kg). Similar results were observed for water intakes during the second hour, but the major effect of treatment with angiotensin II occurred during the first hour (Fig. 1).

*Experiment 2*

Increasing doses of clondine reduced hypertonic saline-induced water intake in a dose-dependent fashion (Fig. 2). The first significant ($p<0.05$) effect occurred at the lowest dose of clonidine administered (6 μg/kg). Similar results were observed for water intakes during the second hour. As with angiotensin II, the major effect of administration of a hypertonic saline load occurred during the first hour after treatment.

*Experiment 3*

Cumulative water intakes of rats administered 3 mg pilocarpine/kg were reduced significantly by both doses of clonidine during the two hours of the experiment (Fig. 3). The first significant effect of clonidine occurred at the lower dose used (6 μg/kg).
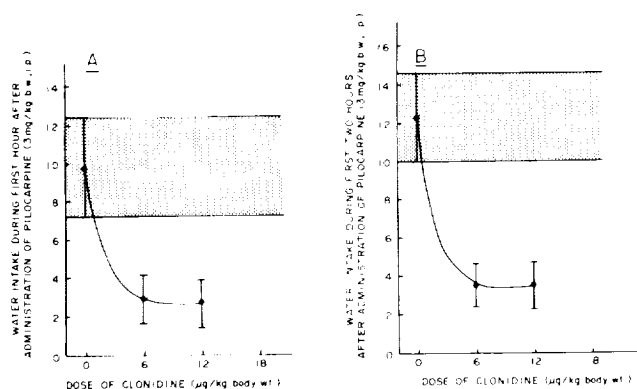
FIG. 3. Water intakes (±SE) of rats during the first (A) and second (B) hours after administration of pilocarpine (3 mg/kg, IP) alone (shaded bar at top of figure) and in combination with 6 and 12 μg clonidine/kg of b.w., IP are shown.

## Experiment 4

Administration of clonidine to dehydrated rats attenuated their water intakes during the first two hours after water was given (Fig. 4). There was a dose-response relationship between dose of clonidine administered and water intake with the maximal effects occurring at 25 and 50 μg/kg of b.w. The first significant reduction in water intake occurred only when 25 μg of clonidine/kg was administered.

### DISCUSSION

The effect of the antihypertensive compound, clonidine, on the dipsogenesis induced in rats by administration of angiotensin II, hypertonic saline and pilocarpine, as well as by a 24 hour period of dehydration, was studied. In all cases, clonidine attenuated water intake. The mechanism by which attenuation occurred is not clear but clonidine also attenuates isoproterenol-induced, as well as 5-hydroxy-tryptophan-induced, drinking in rats [7,19]. Experimental evidence suggests that the dipsogenic response to the latter two compounds is induced ultimately by angiotensin II [3, 4, 10]. Isoproteranol can release renin in vitro [23] while both isoproterenol and 5-hydroxytryptophan also release renin in vivo [3,14]. It is possible that clonidine may have interfered with the drinking responses at this level since it inhibits release of renin [15, 16, 21]. Since clonidine also attenuated the drink induced by angiotensin II, it is unlikely that it blocks only at the level of juxtaglomerular cells and renin release. Clonidine also stimulates α-adrenergic receptors centrally. It seems likely that a central effect of clonidine could also attenuate drinking induced by isoproterenol, angiotensin II, pilocarpine, administration of hypertonic saline and a 24 hour period of dehydration. Le Douarec et al. [13] also reported that clonidine inhibited dehydration-induced water intake in rats but the doses used were much larger.

The inhibition of drinking induced by these diverse stimuli suggests that clonidine may act either directly at thirst centers mediating the drinking response or at nervous pathways beyond those centers. These possibilities are currently under study in this laboratory. Although clonidine at-
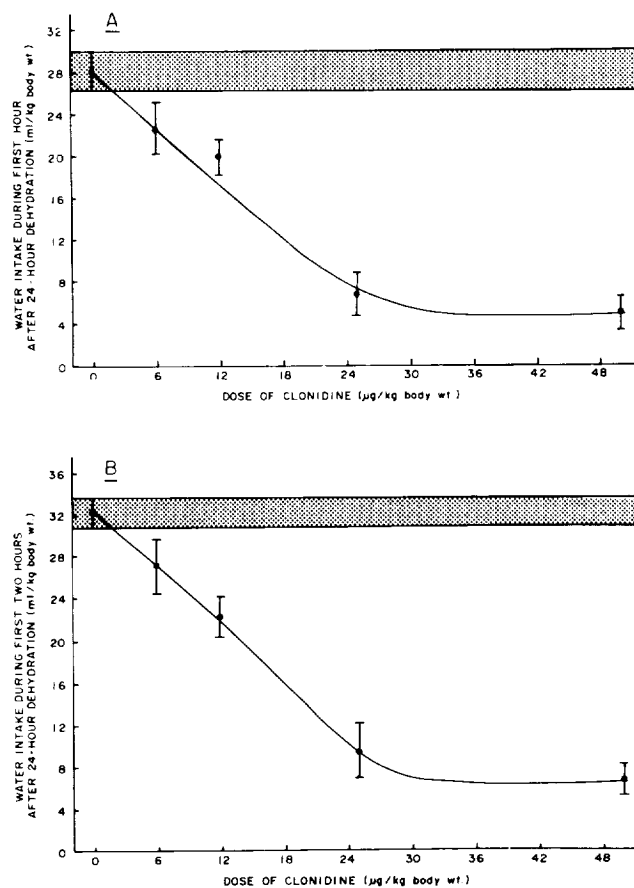


FIG. 4. Water intakes (±SE) during the first (A) and second (B) hours after return of water to rats following a 24 hour dehydration alone (shaded bar at top of each figure) and in combination with 6, 12, 25 and 50 μg clonidine/kg, IP are shown.

tenuated drinking induced by the diverse stimuli listed above, it is especially interesting that it failed to affect water intake significantly when given alone [7]. The studies of Atkinson et al. [1], in which clonidine was administered chronically to rats for 10 days, revealed an initial antidipsogenic effect followed by a secondary dipsogenic effect that could be related to the diuretic effect of the drug.

The inhibition of pilocarpine-induced drinking by clonidine is of special interest. It is not known whether the dipsogenic response to administration of pilocarpine is manifested either peripherally or centrally. A possibility to explain the inhibition is that pilocarpine may act peripherally to initiate the release of renin via its preganglionic stimulatory activity in the sympathetic nervous system. If this is the case, clonidine may also exert its inhibitory effects on pilocarpine-induced drinking peripherally by acting at the level of the renal $\alpha_2$-adrenoreceptors to inhibit the release of renin.

The responses to all dipsogenic stimuli used here were attenuated by the lowest dose of clonidine excepting only the dehydration-induced drinking. In this case, the first significant attenuation of water intake occurred when a dose of 25 μg/kg was administered; i.e., four-fold higher than the dose which attenuated significantly all the other drinking re-

sponses. The fact that dehydration induced the greatest drinking response appears to be consistent with the observation that a four fold greater dose of clonidine was required to inhibit the drinking response than was required for the other dipsogenic stimuli used.

Clonidine is known to exert a central depressant effect when administered to rats [11,20]. The lowest dose used by Laverty and Taylor [11] was 50 μg/kg. Although Tilson *et al.* [20] used lower doses, they observed that the Sprague-Dawley strain was less sensitive to the depressing effects of clonidine than any of the three other strains studied. Thus, 50 μg/kg did not affect spontaneous motor activity in Sprague-Dawley-derived rats while 25 μg/kg induced a significant depression of operant responding activity. Food consumption by rats allowed a 3 hour access to food daily was not affected by acute administration of clonidine until a dose of 200 μg/kg was given [24]. These investigators also reported a reduction in locomotor activity at a dose of 200 μg/kg. It seems unlikely that central depression played a role in reducing the drinking responses reported here since a dose of 6 μg/kg suppressed drinking in all studies except one,

dehydration-induced drinking. A possibility remains that central depression may have played a role in the inhibition of dehydration-induced drinking.

Dry mouth is a common side effect accompanying administration of clonidine to humans [9]. Whether a similar effect occurs in the rat treated acutely with clonidine is not known. If it occurred, the rat failed to respond to treatment by drinking since clonidine alone did not affect water intake significantly [7]. This may also suggest that the effect of clonidine on the central nervous system is either to attenuate the responsiveness of thirst centers or to inhibit nervous pathways beyond the centers mediating drinking behavior. It is apparent that additional studies will be required to clarify this problem.

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